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THE LIFE HISTORY AND MORPHOLOGY OF *SPIROXYS*
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TAXONOMY OF THE NEMATODE GENUS *SPIROXYS* (FAMILY SPIRURIDAE)*

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INTRODUCTION

In connection with a study of the life history and morphology of *Spiroxys contortus* (Rud.) during the years 1929-1932 inclusive I had the opportunity to compare my material collected from the stomachs of various species of North American turtles with specimens of *S. contortus* from Europe. At the same time all other members of the genus available to me were examined and redescribed, the taxonomy of the genus and its member species was revised and appropriate diagnoses were prepared.

HISTORICAL ACCOUNT

Rudolphi (1819: 242-243) was the first to describe a nematode belonging to the present genus *Spiroxys* under the name *Spiroptera contorta* from the stomach of *Emys orbicularis* (Linnaeus) (*Testudinis orbicularis*). He intimated that *Spiroptera contorta* might be synonymous with *Ascaris testudinis*, but stated (1809: 198-199) that he was unable to obtain the appendix by Braun to Schneider's "Naturgeschichte der Schildkröte" (1789) in which a description supporting this name was supposed to appear. Baylis and Lane (1920) also failed to find the reference and designated *Ascaris testudinis* as a *nomen nudum*. Schneider (1866: 125-126) established the genus *Spiroxys* to include only one species, *contorta* R. which he separated from the genus *Spiroptera* R. He failed to change the ending of *contorta* to *contortus* to make it agree in gender with *Spiroxys* with a masculine termination. Indeed, most of the subsequent authors of specific names for mem-

* Contribution from the Zoological Laboratory and the Biological Station of the University of Michigan.

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bers of the genus have likewise used feminine endings with this masculine generic name. Throughout the historical review the original spelling of specific names will be used but elsewhere in this paper, the correct endings will be given.

Seurat (1918) described a nematode from the stomach of an Algerian turtle, *Clemmys leprosa* (Schweigger) and identified it as *Spiroxys contortus* R. Baylis and Lane (1920) described in detail a nematode from the stomach of *Emys orbicularis* under the name of *Spiroxys contorta* R. They pointed out that Seurat's description differed in several fundamental particulars from their material. The exact relation of Seurat's material to other members in the genus will be considered below. Baylis and Lane (1920) described *Spiroxys gangetica* from the stomach of an Indian turtle *Trionyx gangeticus* Cuvier. Baylis and Daubney (1922) added a third species, *S. annulata* from the stomach of another Indian turtle, *Chitra indica* Gray. Morishita (1926) described *Spiroxys japonica* from the small intestines of three species of Japanese frogs, *Rana nigromaculata*, *japonica* and *rugosa*. Walton (1927: 146-147) reexamined Leidy's material from North American turtles and made Leidy's *Ascaris sulcata* Rudolphi and *Ascaris penita* Leidy synonymous with *S. contorta*. He also stated that *Physaloptera contorta* Leidy differed from *Spiroxys* and could not be a synonym of *S. contorta* as suggested by Baylis and Lane (1920). Walton (1927: 147-149) transferred Leidy's *Physaloptera constricta* which was taken from the stomach of *Natrix sipedon* (Linn.) (*Tropidonotus sipedon*) to the genus *Spiroxys*, retaining, however, the specific name. Karve (1928) described *S. torquata* from the stomach of an Indian turtle *Emyda granosa intermedius* Annandale. Cobb (1909) described *S. amydae* from the stomach of *Amyda ferox* (Schneider). Walton (1930) described *S. allegheniensis* from the intestine of *Cryptobranchus allegheniensis* (Daudin).

MATERIAL

Approximately 400 adult *S. contortus* were collected by me from North America turtles. I also had taken *S. contortus* from *Emys orbicularis* (Linn.) from Europe, and *S. amydae* from *Amyda spinifera* (Le Sueur) from Mississippi. Specimens of the following species were borrowed for study: *contortus*, from the British Museum, University of Berlin Museum, and Dr. P. D. Harwood; *amydae*, from the late Dr. N. A. Cobb and Dr. Harwood; *allegheniensis*, from Dr. H. B. Ward, and material identified by Walton as *contortus* and *constricta* from the Leidy collection in the Philadelphia Academy of Natural Sciences.

CLASSIFICATION

The genus *Spiroxys* has been variously placed, by Railliet and Henry (1916) in the Oxyuridae and by Seurat (1918) in the sub-family Camallaninae, family Heterakidae with the genus *Camallanus* Railliet and Henry (1915). Baylis and Lane (1920) created the new sub-family Spiroxyinae, family Gnathostomidae to include only *Spiroxys*. Chitwood and Wehr (1932) followed Baylis and Lane but added *Hartertia* to the Spiroxyinae. Yorke and Maplestone (1926: 302) incorporated the sub-family Spiroxyinae with the genera *Protopirura* Seurat (1914), *Hartertia* Seurat (1914) and *Spiroxys* in the family Spiruridae Oerley (1885). Absence of the cuticular head-bulbs and cervical sacs, characteristic of the Gnathostomes, in *Hartertia*, *Protopirura* and *Spiroxys* and the presence of a glandular esophagus in contrast to the muscular esophagus in the Gnathostomes serve as the principle reasons for retaining these genera in the family Spiruridae. Furthermore it has been proved that *Spiroxys* possesses gubernacula, so this supposed fundamental structural difference between *Spiroxys* and the other two genera disappears (Hedrick unpublished MS.). *Protopirura* may be differentiated from the other two genera by its long vestibule, while *Hartertia* with two very unequal spicules is easily distinguished from *Spiroxys* with its equal to subequal spicules.

Since the life histories of certain species in the three genera are now known, a brief comparison of their life histories in relation to their classification is of interest. Theiler (1919) proved that the termite *Hodotermes pretoriensis* serves as the intermediate host for the bird inhabiting *Hartertia gallinarum*. Leuckart (1867: 112-116) demonstrated the meal worm *Tenebrio molitor* to be the intermediate host for *Protopirura muris* whose adult lives in mice. The essential difference between these two life histories and that of *Spiroxys* is the state of the development of the egg at deposition. The eggs of *Hartertia* and *Protopirura*, parasites of birds and mammals respectively, contain fully developed larvae when deposited but hatching does not take place until the eggs are eaten by the intermediate host, thus enabling the larvae to survive in a dry environment. Eggs of *Spiroxys* leave the definitive host in very early cleavage stages and soon perish unless they are in water where they incubate and hatch. The newly emerged larvae is free living and is attached to some object in the water with its sheath. It is quite apparent that this difference in degree of egg development and place of hatching is a correlative adaptation to the differences in the habits of life of the definitive hosts.

SPECIFIC CONSIDERATIONS

S. contortus. The nematodes collected by me from turtles were determined after careful comparison with material from England and

Germany to be *S. contortus*. Although these borrowed specimens were old and poorly fixed careful study revealed several fundamental structural characters such as cuticular collar with spines, colorless gubernaculum, and median genital papilla which had been overlooked in earlier studies. The finding of these structures removed the last doubt that my material was conspecific with the European *S. contortus*. This species has been redescribed by Hedrick (unpublished MS.).

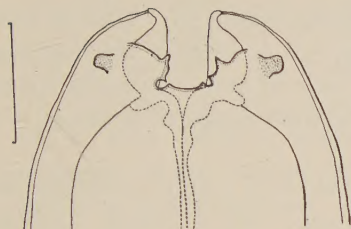
S. amydae. Examination of type specimens of *Spiroxys amydae* Cobb (1929) from the stomachs of *Amyda ferox* (Schneider) loaned to me by the late Dr. N. A. Cobb of the U. S. Department of Agriculture, specimens loaned by Dr. Harwood of Vanderbilt University and material collected by the author showed that these nematodes are very similar to the description of *S. gangeticus* Baylis and Lane (1920) except that adults of *S. amydae* are considerably smaller than those of *S. gangeticus*. A gubernaculum, cuticular collar with two dorsal and two ventral spines and one lateral spine, a median genital papilla in the male, and the two dorso-lateral caudal papillae on the tail of the female were also found in this species (Figs. 3, 4, 8, 9 and 10). The amphids reported for *S. amydae* by Cobb (1929) could not be detected in an *en face* view of a mounted head loaned by Dr. Cobb, although it was studied under an 1.8 mm. homogeneous immersion lens. My studies upon well preserved specimens of *S. amydae* show that the crenations near the posterior of the body reported for *S. amydae* by Cobb are not true characters but merely artifacts caused by poor preservation. The long narrow tail of the female *S. amydae* is quite distinctive.

Measurements of *S. amydae* in millimeters: lips, length, males, .061 to .064; females, .056 to .064; thickness, males, .109 to .119; females, .097 to .129. Length of body, males, 23 to 32; females, 26 to 37.

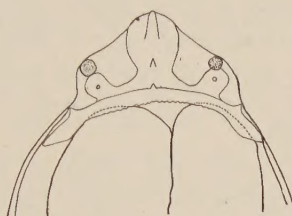
EXPLANATION OF PLATE

All the drawings were made with the aid of a camera lucida or a micro-projection apparatus; the details were filled in at the same magnification. The reference lines represent a length of one-tenth millimeter.

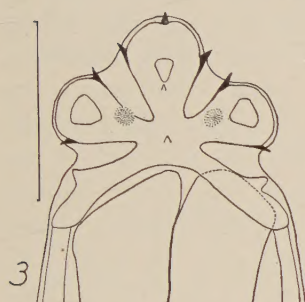
- Fig. 1. *S. allegheniensis*. Dorsal view of head with very small dorsal lobes of the lips.
 Fig. 2. *S. allegheniensis*. Lateral view of head, same magnification as Fig. 1.
 Fig. 3. *S. amydae*. Lateral view of head.
 Fig. 4. *S. amydae*. Dorsal view of head, same magnification as Fig. 3.
 Fig. 5. *S. constrictus*. Lateral view of head, same magnification as Fig. 3.
 Fig. 6. *S. constrictus*. Gubernaculum, lateral view, same magnification as Fig. 3.
 Fig. 7. *S. constrictus*. Dorsal view of head, same magnification as Fig. 3.
 Fig. 8. *S. amydae*. Posterior end of female, lateral view showing caudal papilla.
 Fig. 9. *S. amydae*. Gubernaculum and end of spicule, lateral view.
 Fig. 10. *S. amydae*. Tail of female, ventral view showing caudal papillae.



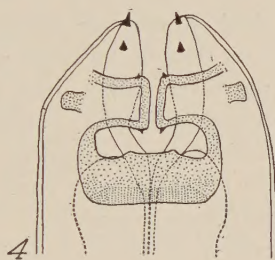
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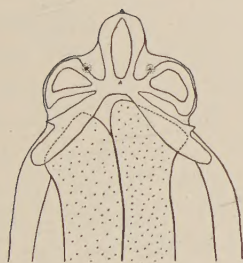
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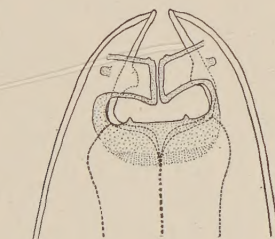
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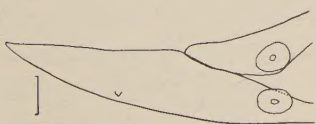
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Diameter of body, males, .372 to .56; females, .38 to .59. Tail of males moderately long, .232 to .264 mm. long; tail of females long and narrow, .36 to .48 mm. long and .18 mm. wide at anus. Spicules robust, about .72 to 3.37 mm. long. Distance between cuticular striations 5 to 11 micra.

S. constrictus. Walton (1927) reexamined Leidy's material and allocated *Ascaris penita* Leidy (1886) and *Ascaris sulcata* Rudolphi (1819) Leidy (1887) to the genus *Spiroxys*, species *contortus*. He also transferred to this genus specimens that Leidy found in the stomach of *Natrix* and had named *Physaloptera constricta* Leidy (1856). After a careful study of Leidy's specimens of these three species, it is my opinion that all of these worms are conspecific and that they belong in the genus *Spiroxys* as Walton indicated, but to the species *constrictus*, not *contortus*. Of the three names used by Leidy, *constrictus* is the only one available because Rudolphi's species *A. sulcata* clearly belongs to the superfamily Ascaroidea. Rudolphi described the head of this species as distinct and provided with three large lips, "*Caput* valvulis tribus majusculus instructum, discretum." In contradistinction to this, the same author described the head of *Spiroptera contorta*, a true *Spiroxys* and Spiruroid thus, "*Caput* saepe tres tantum papillas in trifolii modum positas exhibet." *Constricta* Leidy (1856) has priority over *penita* Leidy (1886).

The worms of the existing Leidy collection examined by me are as follows: three male and three female specimens of *sulcata*, most of these immature, two male specimens of *constricta*, and fragments of female *penita*. The *sulcata* and *constricta* material was poorly preserved but, as intimated above, the *penita* material was much worse. Since these worms are of the same species and they are so few in number, the description and measurements below are a composite of the three, rather than of any one of the former species. These worms are slender and rather delicate. The most distinctive character is the posterior slanting of the dorsal and ventral lobes of the lips, that is the posterior margin of the dorsal and ventral lobes forms an acute angle with the longitudinal body axis instead of an approximate right angle, as is true in the other known species (Figs. 5 and 7). The cuticular support for the lips is delicate and the arch of the cuticular collar appears to be high. The spicules are very slender and have filiform pliable terminations. They are cross striated despite Walton's statement to the contrary in regard to *constrictus*. There is a definite gubernaculum which possesses less cuticular material than the gubernaculum of *contortus* (Fig. 6).

Measurements of *S. constrictus* in millimeters: lips, length, .048 to .087; thickness, .073 to .10; distance of cervical papillae from anterior

tip of head, .09 to 1.34; body diameter, .217 to .480; body length, 15 to 24; tail length, .17 to .25; spicule length, 1.82 to 2.45, and distance between striations, .003 to .006.

The fact that the specimens known to Leidy as *Physaloptera constricta* were found in snakes should not form a basis for the differentiation of this species, because *Natrix* is probably not the normal host for these nematodes. It seems to me that Walton correctly interpreted the situation in these words, "The Watersnakes living in a similar environmental condition, are perhaps not the original or normal host of this species . . . but certainly can act as satisfactory substitute hosts when accidentally infected." This is all the more plausible since it is probable that fish, tadpoles and frogs serve as the second intermediate hosts for *S. constrictus* as well as for *S. contortus*.

S. allegheniensis. The study of two male specimens of *S. allegheniensis* has revealed several fundamental structures overlooked by Walton (1930). The head appears very short and wide because the usual cervical constriction is absent. The dorsal and ventral lobes of the lips are greatly reduced, especially the cuticular elements supporting them (Figs. 1 and 2). The cuticular collar is narrow and broadly arched, the posterior margin of the arch irregular. The submedian papillae, lateral papillae, cervical spines, and lateral cuticular spines are prominent. The esophagus is stout and appears to be short, but is only relatively so because of its large diameter. The spicules are robust and cross-striated. The gubernaculum is reduced to a cuticular thickening of the curved spicule sheaths in the cloacal region. There is a definite median genital papilla. The cuticular striations are very fine.

Measurements in millimeters: lips, thickness, .14, length, .087 to .089; nerve ring to tip of head, .77; esophagus, length, 2.52, maximum diameter, .357; body length, 24 to 27; body diameter at nerve ring, .388; at end of esophagus, .775; at middle of body, .884; and at vesicular swelling, .667; tail length, .37, and spicule length, 1.7 to 2.0.

S. algericus n. sp. The nematode identified by Seurat (1918) as *Spiroxys contortus* from the stomach of *Clemmys leprosa*, an Algerian turtle, is said by Baylis and Lane (1920) to differ from their forms of *S. contortus* as follows:

- "(1) the presence of an internal tooth on *each* lobe of each lip;
- (2) the presence of a pair of large papillae on the middle lobe of each lip, instead of one small papilla;
- (3) the presence of an adanal, lateral pair of genital papillae in the male;
- (4) the presence of an accessory piece in the male."

Seurat's original description was carefully compared by me with specimens of *S. contortus* from the British and Berlin Museums and

in my opinion the interpretation of Baylis and Lane with respect to items one and two is correct. As a result of this comparison another difference can be stated. This is that the tail of the male in Seurat's form is 612 micra long as compared with the maximum length of 388 micra for the tail of an adult male *S. contortus*. It has been pointed out previously in this paper that the presence of a gubernaculum (accessory piece) in both groups is a point of similarity and not a difference. Reference has also been made by the author to the pair of adanal lateral genital papillae, indicating that this pair may be pair number 6, normal to the genus. The location of this pair number 6 is rather variable with respect to the anus, being sometimes posterior, at other times parallel, or anterior. As Seurat mentioned only 11 pairs in all, it is my opinion that this adanal lateral pair of genital papillae may be substituted for the normal pair, number 6.

Since Seurat is an experienced worker and a good observer, it must be assumed that his description is accurate. Therefore, the other differences, namely, the presence of an internal tooth on each lobe of each lip, the presence of a pair of large papillae on the middle lobe of each lip instead of one small papilla, and the long tail in the male instead of a tail of moderate length, constitute characters for a new species. These differences are so marked that a new name, *Spiroxys algericus* is proposed for this Seurat material, although it was impossible for me to study the specimens. However, if further study of this material should prove that it is really *S. contortus*, then *S. algericus* can be reduced to synonymy.

Measurements in millimeters for *S. algericus* from Seurat's description are: total length, 31 mm.; thickness, .60 mm.; nerve ring to anterior end, .47 mm.; cervical papillae to anterior end, .625 to .66 mm.; excretory pore to anterior end, .815 mm.; esophagus length, 2.74 mm.; tail length, .612 mm.; spicules equal, length, 3.63 mm., and gubernaculum length, .120 mm. All measurements are from male specimens.

DIAGNOSES

The finding of a gubernaculum in the males of the species of *Spiroxys* prevents the use of that structure as a character for the differentiation of *Spiroxys* from *Hartertia* and *Protospirura* and makes it necessary to amend the diagnosis given by Yorke and Maplestone (1926: 302) for the sub-family accordingly.

Sub-family Spiroxyinae Baylis and Lane (1920).

"Spiruridae: mouth with large distinctly trilobed lips; the cuticle of the inner surface is thickened and thrown into folds and tends to interlock with that of the outer lip; vestibule present without ring-like or spiral thickenings; cephalic and esophageal regions not ornamented with cuticular plaques." Gubernaculum present.

Type genus, *Spiroxys* Schneider (1866).

Other genera, *Hartertia* Suerat (1914) and *Protospirura* Seurat (1914).

Genus *SPIROXYS* Schneider (1866) emend.

Generic diagnosis. Spiroxyinae: head with two prominent trilobed lips; cuticle of median lobe thickened and usually projecting anteriorly to form blunt tooth; dorsal and ventral lobes tending to oppose those of opposite lip; each lip bearing two sub-median papillae and one small lateral papilla, rarely only two papillae on each lip, those on the median lobe. Cuticular collar usually with six spines, two dorsal, two ventral and one small spine on each lateral surface, rarely one dorsal and one ventral spine. Vestibule with cuticular lining, short, leading into elongate, club-shaped esophagus. Esophagus divided into two regions, anterior part short and muscular, gradually merging into longer glandular part. Male: caudal alae well developed, with preanal vesicular swelling; eleven pairs of papillae, four preanal and seven postanal; seventh and eighth pairs sessile, situated anterior and posterior to the anus respectively; one small median genital papilla anterior to the anus; spicules long, slender, cross-striated and equal to sub-equal; gubernaculum uncolored. Females: vulva near middle of the body; annulated vagina directed forward; two opposed, bi-pouched uterine branches in mature worm; two dorso-lateral caudal papillae on tail. Eggs pitted, deposited in one or two celled stage. Larvae, free-living, and sheathed. First intermediate host, Cyclops. Adults parasitic in stomach wall of turtles and snakes, and intestines of amphibia.

Type species: *Spiroxys contortus* (Rudolphi, 1819).

Synonyms:

Ascaris Rudolphi (1809: 193; 1819: 25 and 242) *e. p.*

Spiroptera Rudolphi (1819: 25 and 242) *e. p.*

Physaloptera Leidy (1856: 53) *e. p.*

Spiroxis Schneider (1866: 29) (misprint).

SPECIFIC DIAGNOSES

S. constrictus (Leidy, 1856) Walton (1927). Slender delicate *Spiroxys*. Males 17.5 to 23.5 mm. long, .21 to .326 mm. broad, females (only young specimens observed) 9.6 to 13.4 mm. long. Cuticular striations 3 to 6 micra apart. Tail short, length in males .171 to .187 mm., in females .25 mm. Spicules delicate, 1.82 to 2.45 mm. long, with filiform pliable terminations. Gubernaculum difficult to differentiate. Arch of cuticular collar high, cuticular supports for lips delicate. Dorsal and ventral lobes of lips slanting posteriorly, forming acute angles with median lobe. Lips, .048 to .087 mm. long and .073 to .10 mm. thick. Parasites in Leidy's collection from the stomachs of *Chrysemys picta* and *Natrix sipedon*.

Synonyms:

Physaloptera constricta Leidy (1856: 53).

Ascaris penita Leidy (1886: 312).

Ascaris sulcata Rudolphi (1819), Leidy (1887: 21-22).

Spiroxys contorta (Rud., 1819), Walton (1927: 146-147) *e. p.*

S. contortus (Rud., 1819), Schneider (1866). *Spiroxys* of medium size, males 19.5 to 26.6 mm. (av. 23.5 mm.) long, females 22.5 to 39.8 mm. (av. 27.7 mm.); maximum diameter of males .30 to .39 mm., of females .45 to .57 mm. Cuticular striae 5 to 6 micra apart. Tail length moderate, in males, .233 to .388 mm., in females .18 to .45 mm. Spicules sharply pointed, 1.85 to 3.5 mm. long. Gubernaculum prominent, .108 to .110 mm. long. Arch of cuticular collar moderate, cuticular spines prominent, cuticular supports for lips moderate to heavy. Dorsal and ventral lobes of lips at right angles with median lobe. Lips of males .059 to .08 mm. long, .087 to .10 mm. thick; of females .07 to .122 mm. long, .09 to .13 mm. thick. Parasites in the stomachs of various species of turtles.

Synonyms:

Spiroptera contorta Rudolphi (1819: 242-243).

Spiroxys contorta (Rud., 1819), Schneider (1866: 125).

Spiroxys contorta Von Linstow (1909: 58).

Spiroxys contortus Railliet and Henry (1916: 114).

S. algericus n. sp. *Spiroxys* of moderate size, males 31 mm. long, and .60 mm. maximum diameter. Cuticular striations 7 micra apart. Spicules slender, equal, 3.63 mm. long. Lips three-lobed, each lobe bearing one small internal tooth, median or lateral lobes very large, each with pair of large papillae. Parasites in stomach wall of *Clemmys leprosa*, an Algerian turtle.

Synonym:

Spiroxys contortus (Rud., 1819), Schneider (1866), Seurat (1918: 23-25).

S. japonicus Morishita (1926). *Spiroxys* thick or stout, males 14 to 22 mm. long, females 18 to 31 mm.; maximum diameter males .46 to .64 mm., females .71 to .98 mm. Cuticular striations fine. Length of tail moderate, .33 to .37 mm. in males, .22 to .32 mm. in females. Spicules blunt, 2 to 2.2 mm. long. Lips of males .125 mm. long, .225 mm. thick, of females .15 mm. long, .36 mm. thick. Apparently only one spine on dorsal and ventral ends of cuticular collar. Cuticular annulations confined to anterior end of body. Parasites in duodenum of frogs, *Rana nigromaculata*, *japonica*, and *rugosa* in Japan (Morishita, 1926) and in *R. nigromaculata* in China (Hsü and Hoeppli, 1931).

S. torquatus Karve (1928). Large stout *Spiroxys*, males 19 to 36 mm. long, females 27 to 39 mm.; maximum diameter, male .277 to .66 mm., females .6 to .91 mm. Cuticular striations coarse, 17 to 18 micra distant. Tail moderately long, .21 to .3 mm. in males, .52 to .54 mm. in females. Spicules 2.25 mm. long, finely pointed. Arch of cuticular collar moderate to small. Dorsal and ventral lobes of lips at right angles with median lobe. Lips of males .075 to .09 mm. long and .112 to .15 mm. thick, of females .097 mm. long and .17 to .20 mm. thick. Parasites in stomach of *Emyda granosa intermedius* Annandale, an Indian turtle, and stomach of *Trionyx cartilagineus*, a Maylan turtle, Baylis (1933).

S. annulatus Baylis and Daubney (1922). *Spiroxys*, of medium size, males 20 to 25.4 mm. long, females 30 to 34 mm.; maximum diameter males .5 to .6 mm., females .8 to .85 mm. Cuticular striations very coarse, striae 25 micra apart. Tail very short, about .2 mm. long in both sexes. Spicules slender, sharply pointed, 1.6 to 2.3 mm. long. Arch of cuticular collar moderate. Dorsal and ventral lobes of lips at right angles with median lobe. Lips .06 mm. long and .016 mm. thick. Parasites in stomach of *Chitra indica*, an Indian turtle.

S. allegheniensis Walton (1930). *Spiroxys* stout, males 24 to 27 mm. long, maximum diameter .85 to .9 mm. Cuticular striations very fine. Tail length moderate, in male .36 to .37 mm. Spicules robust and short, 1.7 to 2 mm. long. Gubernaculum reduced. Cuticular collar delicate, posterior margin rough, only slightly arched. Dorsal and ventral lobes of lips extremely small, difficult to detect in dorsal view of head. Lips, .087 to .089 mm. long, .14 mm. thick. Males only described. Parasites in the small intestine of hellbender, *Cryptobranchus allegheniensis*.

S. amydae Cobb (1929). *Spiroxys* of moderate size, males 23 to 32 mm. long, females 26 to 37 mm.; diameter, males, .372 to .56 mm., females .38 to .59 mm. Cuticular striations 5 to 11 micra. Tail of males moderately long, .232 to .264 mm.; of female long and narrow, .36 to .48 mm. long and .18 mm. wide at anus. Spicules robust, about 2.72 to 3.37 mm. long. Gubernaculum prominent. Cuticular collar moderately arched. Dorsal and ventral lobes of lips at right angles with median lobe. Each lip with special armature of six, fine sharp teeth, one on either edge of each lobe; cuticular tooth at apex of median lobe, sharp. Lips of male .061 to .064 mm. long, .109 to .110 mm. thick, of females .056 to .064 mm. long, .097 to .129 mm. thick. Parasites in stomachs of *Amyda ferox* (Schneider) and *A. spinifera* (Le Sueur).

S. gangeticus Baylis and Lane (1920). Large stout *Spiroxys*, males 39 to 43 mm. long, females 48 to 50.3 mm.; diameter, males .75 mm., females .85 to .95

mm. Cuticular striations moderately coarse, 8 to 17 micra apart. Tail long, .35 mm. in males; .65 mm. in females. Spicules robust 2.1 to 2.25 mm. long, ending in blunt points. Cuticular collar moderately arched. Dorsal and ventral lobes of lips at right angles with median lobe. Each lip carries special armature of six fine sharp teeth, one on either edge of each lobe; cuticular tooth at apex of median lobe sharp. Lips of males .14 to .16 mm. long and .11 to .13 mm. thick, of females .16 to .2 mm. long and .13 mm. thick. Parasites in stomachs of *Amyda gangeticus* (*Trionyx gangeticus*), an Indian turtle.

GEOGRAPHICAL DISTRIBUTION

The nine species of *Spiroxys* are distributed as follows: *annulatus* and *gangeticus*, India; *torquatus*, India (Karve, 1928) and Malay (Baylis, 1933); *japonicus*, Japan and China; *algericus*, Africa (Algeria); *contortus*, Europe and the United States of America, and *constrictus*, *allegheniensis*, and *amydae*, the United States.

Although four species have been found in North America, their actual distribution is poorly known. *S. allegheniensis* was taken from *Cryptobranchus allegheniensis*, which is restricted to the streams of the Allegheny Mountains. This host is limited in range, but it seems likely that some member of the genus *Spiroxys* will be found in frogs and salamanders whose distribution in America is quite extensive. The forms now known as *S. constrictus* were probably collected from hosts found in the eastern part of the United States. No recent data are available in regard to the distribution of the two species above, as all of the worms known to be in the species *S. constrictus* were collected by Leidy before 1890 and the material called *S. allegheniensis* was collected in 1909.

The distribution of *S. contortus* in America has not been considered until recently. For this reason, the known range for this species in the United States is Oklahoma and Texas (Harwood, 1931 and 1932) and Michigan, Louisiana, Wisconsin and Washington as determined by the writer. Since this species inhabits a variety of genera and species of turtles, its distribution in America is probably much more extensive than indicated above. Its rather wide distribution in Europe and America may indicate that it is an old form and that in ancient times the ancestors of the present hosts for *contortus* occupied the same geographical territory.

The known range for *S. amydae* is confined to the type locality, the Mississippi River, Texas as reported by Harwood (1932) and the state of Mississippi as determined by me. It is of interest to note that *Amyda gangeticus* (*Trionyx gangeticus*) in India is infected with *S. gangeticus*, which is very similar to *S. amydae*, differing only in size, the Indian worm being the larger.

Thus species of *Spiroxys* have been found in only a few isolated areas of the world. Nothing is known of their presence in Central or

South America, West Indian Islands, New Zealand, Australia, the East Indian Archipelago, in most of Asia, in most of Africa, and in the greater part of the United States. Because this parasite genus is known to inhabit many host species, in the groups Reptilia and Amphibia, and because of the possible discovery of a greater variety of hosts, it may be predicted that members of the genus *Spiroxys* will be found in nearly every part of the tropical and temperate regions of the earth.

SUMMARY

The genus *Spiroxys* has been reviewed and specimens of several species have been carefully studied. The following structures, that either had not been previously described or had been regarded as specific in rank, have been established as generic characters: gubernaculum, cuticular collar with dorsal and ventral spines and a small spine on each lateral surface, median genital papilla in the male, and the two dorso-lateral papillae on the tail of the female.

The nematodes, *Ascaris sulcata* R. Leidy and *Ascaris penita* Leidy, which Walton placed under *S. contortus*, have been shown to be conspecific with *S. constrictus* (Leidy, 1856), Walton (1927). This species has been redescribed.

The nematodes described by Seurat (1918) as *S. contortus* has been found to be a new species and was named *S. algericus*.

Specific diagnoses for the nine recognized species of *Spiroxys* have been presented, some of them revised.

The present knowledge of the geographical distribution of the species of *Spiroxys* has been given.

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THE LIFE HISTORY AND MORPHOLOGY OF *SPIROXYS*
CONTORTUS (RUDOLPHI); NEMATODA: SPIRURIDAE*

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INTRODUCTION

Coiled nematodes which live threaded into the stomach mucosa of turtles have been known for more than a century, but no clearly recognizable description was made of them until within the last fifteen years. Similar worms from the stomach of a snake and intestines of frogs and *Cryptobranchus* have been described in the past decade. Concerning the life history of these worms nothing has been written.

These nematodes belong to the genus *Spiroxys* Schneider (1866), sub-family Spiroxyinae Baylis and Lane (1920), family Spiruridae Oerley (1885), and super-family Spiruroidea Railliet and Henry (1915) according to the classification of Yorke and Maplestone (1926). The sub-family Spiroxyinae also includes the genera *Protospirura* Seurat (1914), living in mammals, and *Hartertia* Seurat (1914) in birds.

Spiroxys from the stomach of turtles in Michigan were compared, and found to be con-specific, with specimens of *contortus*, the type species of

* Contribution from the Zoological Laboratory and the Biological Station of the University of Michigan.

the genus, from the stomach of the European turtle. Specimens of other species from different regions were also examined.

The life history of *Spiroxys contortus* has been studied in detail and has been found to present new features not exhibited in any other known life history of a spiruroid living in either an aquatic or terrestrial host. Previously but few nematodes had been proved to undergo development to the infective stage in the body cavity of cyclops. *Dracunculus medinensis* is one of these few.

The study of the morphology and life history of *Spiroxys contortus* was begun during the summer of 1929. Most of the experimental work was performed during the summers of 1929 to 1931 inclusive at the Biological Station of the University of Michigan and the fall and winter of 1931 at the University of Michigan.

I wish to express my sincere appreciation to Dr. George R. La Rue, under whose direction this work was completed, for his criticisms and helpful suggestions. To Dr. L. J. Thomas my thanks are due for his interest and valuable suggestions at the inception of the problem and during the progress of the work in the two succeeding summers.

I am greatly indebted to the following for the loan of material: the British Museum of Natural History, The University of Berlin Museum, the late Dr. N. A. Cobb, Dr. P. D. Harwood, Dr. H. B. Ward and Dr. Percy B. Moore for some of Leidy's material from the Philadelphia Academy of Natural Sciences. I am grateful to Dr. Stillman Wright for aid in the identification of the cyclops, to Mrs. Helen T. Gaige for aid in the identification of turtles, and to Dr. Werner Schröder for supplying living specimens of the European turtle, *Emys orbicularis*.

TECHNIQUE

Young and adult nematodes were studied alive, in cleared whole mounts and by means of sections. All measurements of the larvae were made from living material by measuring with a map measurer the image projected on a screen by a micro-projection apparatus at a determined magnification. In order to detect larval forms which live in cysts or free in the stomach tissue, the stomach wall was firmly compressed between two rather large glass plates.

The best fixing and killing fluid for these worms is a mixture of 5 per cent formalin and a saturated solution of mercuric chloride in the ratio of 9:1, used hot. This does not produce blisters, kills the worms relatively straight, causes but little shrinkage and permits the making of good histological sections. In preparation for preservation the worms were washed in grades of alcohol 35, 50, and 70 per cent, then treated with iodine dissolved in 70 per cent alcohol. Infected cyclops were fixed in the formol-corrosive, or better, by the use of fumes from a 2 per cent aqueous solution of osmic acid.

Liquid phenol (phenol 88 parts, water 12 parts) proved to be the most useful and convenient medium for examination *in toto*. The Spiroxys studied are two to four centimeters long and clear in approximately fifteen minutes after they are transferred directly from 70 per cent alcohol into the phenol solution. This process does not cause collapse, and it renders the worms so limp that they may be rotated into any position desired. Too great transparency which makes the outline of some structures difficult to determine is easily remedied by the addition of water. Light green or orange G stains are

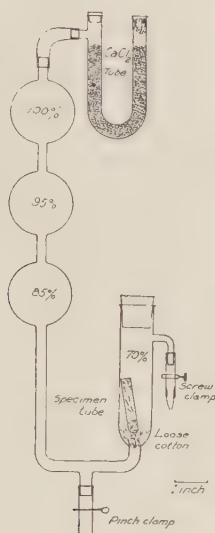


FIG. 1. Dehydrator, bulb type.

also used to advantage in the phenol solution. After completing the study the worms are washed in 70 per cent alcohol containing a trace of potassium acetate. The latter neutralizes the phenol and prevents the preserved material from turning brown. Caution should be exercised in the use of the phenol because of the following effects: too long exposure of material to the solution causes maceration; direct contact with human skin produces a caustic reaction; the phenol destroys the lacquer finish on microscopical equipment.

Total permanent preparations were mounted in thick cedarwood oil, damar, or Farrant's gum arabic medium. The nematodes were gradually brought into the Farrant's medium from either water or glycerine. In order to mount the worms in cedarwood oil or damar the nematodes must be gradually brought into absolute alcohol. A dehydrator of the bulb type was used for this purpose. This apparatus consists of a magazine tube which contains the specimens, a U tube reservoir, and three bulbs in a vertical series attached to the upper end of the U tube (text figure 1). The magazine

and reservoir is filled with 70 per cent alcohol, the lowest bulb with 85 per cent alcohol the next with 95 per cent alcohol, and the upper with absolute alcohol. A calcium chloride tube is attached to the bulb containing absolute alcohol. The flow of alcohol through the apparatus may be regulated by a screw clamp on a side arm attached near the upper end of the magazine tube. Nematodes which are not straight should be tied between two small pieces of glass before they are placed in the magazine tube. In order to take specimens gradually into cedarwood oil Martini's calcium chloride desiccator method was used. The worms were transferred from absolute alcohol into a solution of 10 or 20 per cent cedar oil dissolved in absolute alcohol. When a dish containing this solution and the worms was placed in the desiccator, the alcohol gradually evaporated and left the nematodes in concentrated cedar oil. Sufficient fluid must be used so that when the evaporation is completed, the worms will be entirely covered because air bubbles will permeate the worms if cedar oil does not completely cover the specimens.

Von Peterfi's method (Romeis, 1928:91), of paraffin-celloidin imbedding by the use of celloidin dissolved in methylbenzoate was employed in preparing the worms for sectioning. Since the adults are 2 to 4 cm. long, they were cut into small pieces before dehydration. Sections were stained with Mayer's haemalum or Mayer's acid haemalum and eosin.

The methods used in the experimental work will be outlined when their respective applications are considered.

MATERIAL

The natural definitive hosts of *Spiroxys contortus* in North America are: *Chrysemys bellii marginata* (Agassiz), *Emys blandingii* (Holbrook), *Graptemys geographica* (Le Sueur), *Sternotherus odoratus* (Latreille), *Kinosternum subrubrum* (Lacépède), *Chelydra serpentina* (Linnaeus), *Pseudemys texana* Baur, and *Pseudemys elegans* (Wied). *S. contortus* was also found by me in *Emys orbicularis* (Linn.) from Europe. As experimental final hosts *Terrapene carolina carolina* (Linnaeus) and *T. carolina ornata* were used.

Experimentally infected first intermediate hosts are: *Cyclops leuckarti* Claus (1857) (*Mesocyclops obsoletus*) (Koch), *Cyclops albidus* Jurine (1820) (*Macrocyclops annulicornes*) (Koch), *Cyclops serrulatus* Fischer (1851) (*Leptocyclops agilis*) (Koch), and *Cyclops brevispinosus* Herrick (1884).

The second intermediate hosts in which encysted larvae have been discovered are: *Umbra limi* (Kirtland), young *Ameiurus nebulosus* (Le Sueur), tadpoles and adults of *Rana clamitans* Latreille, larvae and adults of *Triturus viridescens* Rafinesque, and dragonfly nymphs, probably of the genus *Sympetrum*.

Most of the turtles used in this investigation were collected from four general regions in Michigan: Douglas lake and vicinity in Cheboygan and

Emmet counties, Fife Lake in Grand Traverse county, Muskegon county, Washtenaw and adjacent counties.

Infected second intermediate hosts were collected principally from Fontinalis Run, a bog swamp on the northeast shore of Burt Lake, Cheboygan county; pools adjacent to Elk Lake, Antrim county; channels entering Fife Lake; a mill race along the Huron River west of Ann Arbor, and a clay-bottomed pool near the village of Platt, east of Ann Arbor.

LIFE HISTORY

The life history of *S. contortus* has been experimentally completed through the following stages: the incubation and hatching of the eggs into sheathed free-living larvae; development in artificially infected first intermediate hosts (certain species of cyclops); the infection of second intermediate hosts (tadpoles and *Ameiurus*); the introduction of the larvae from both the first and second intermediate hosts into the final hosts (various species of turtles) and the growth of the larvae into adults. Each phase of this life history has been studied in detail and will be considered *seriatim*.

Eggs and Their Development

The adult females were permitted to deposit their eggs in Syracuse watch glasses containing tap water. After egg-laying, the worms were removed and the water changed every twenty-four hours to reduce bacterial growth.

The egg shell is thin and the ovum soon dies unless the egg is in water. Development of the shell occurs in that part of the second uterine pouch which is close to the oviduct. The shell is pitted, but the opacity of the inner undivided protoplasm (fig. 14) causes the shell to appear stippled with small curved lines. During cleavage the egg becomes more transparent, and the pits in the shell become obvious (fig. 15). Fifty eggs from different females measured in length .055 to .073 mm. (av. .065 mm.) and in width .039 to .05 mm. (av. .044 mm.).

Eggs leave the female in the one- or two-celled stage. Eggs in the one-celled stage are deposited at times, and I have also observed eggs in the two-celled stage within the uterus and vagina; when they leave the turtle they are in the four- to sixteen-celled stage. In experiments to determine the incubation period of eggs in correlation with temperature eggs from the same female were separated into three groups. One group was left at room temperature; another was placed in an electric refrigerator, and the third in an incubator. The state of development of each group was observed every twelve hours and the temperatures registered by maximum and minimum thermometers were recorded at the same time.

At room temperatures, 23°C. to 25°C., the stages of the eggs at different times were: 12 hours, morula, blastula, and early gastrula; 24 hours, early

tadpole; 36 hours, late tadpole, movement of embryo had begun; 48 hours, early worm; 60 hours, worm; 72 hours, advanced worm; and at 84 hours, hatching had commenced. Hatching continued from three to four days, but some eggs did not hatch although fully embryonated.

In the refrigerator, at 10°C., eggs were in the late tadpole stage by the eighth day but developed no further although they remained in the refrigerator for 15 days. When they were placed at room temperatures, hatching began in 36 hours.

In the incubator, at 37°C., eggs did not attain the well developed tadpole stage until the fifth day and remained in this stage although they were kept in the incubator for twelve days more. When moved to room temperatures, they began to hatch in 24 hours. Water was changed daily to avoid excessive growth of bacteria and protozoa.

The above experiments indicate that at an optimum temperature, 20 to 25°C., about four days of incubation is required, but at temperatures as high as 37°C. or at cooler temperatures, 8–12°C., development is considerably retarded. However, when these eggs are brought to optimum

EXPLANATION OF PLATE XLIX

All of the drawings were made with the aid of a camera lucida or a micro-projection apparatus; the details were filled in at the same magnification. The reference lines represent a length of one-tenth millimeter unless otherwise labelled.

Spiroxys contortus

- FIG. 1. Adult head, throat view.
- FIG. 2. Adult head, lateral view; *cc* cuticular collar; *cs*, cuticular spine; *lcs*, lateral cuticular spine; *lp*, lateral papilla; *smp*, sub-median papilla, and *t*, tooth.
- FIG. 3. Adult head, dorsal view; *cs*, cuticular spine, *lp*, lateral papilla, and *smp*, sub-median papilla.
- FIG. 4. Diagram of a cuticular collar in an adult.
- FIG. 5. Head of a 2.2 mm. larva, lateral view.
- FIG. 6. Head of a 2.2 mm. larva, dorsal view, *smp*, sub-median papilla.
- FIG. 7. Eight-day-old larva from cyclops, lateral aspect, *lt*, larval tooth.
- FIG. 8. Esophagus, sagittal sections of middle and posterior regions, glandular part represented by stipple work.
- FIG. 9. Protrusion on larval tail.
- FIG. 10. *Cyclops leuckarti* with three larval nematodes, 16 days after infection.
- FIG. 11. Five-day-old larva from cyclops.
- FIG. 12. Free-living larva with sheath.
- FIG. 13. Reproductive organs of a mature female, *od*, oviduct; *upa*, uterine pouch anterior; *upb*, first uterine pouch posterior; *upp*, second uterine pouch posterior; *v*, vulva, and *va*, vagina.
- FIG. 14. Egg when first deposited.
- FIG. 15. Pitted egg shell after hatching.
- FIG. 16. Posterior end of the female worm, *cp*, caudal papilla.
- FIG. 17. Fully developed larva from the body cavity of cyclops, *gp*, genital primordium.



PLATE XLIX

temperatures, growth proceeds rapidly and the eggs soon hatch. Other factors in addition to temperature no doubt, also affect the length of incubation. One of these is vitality of the eggs. Eggs deposited by different females on the same day and kept under the same conditions may hatch at different times. In some instances hatching of the eggs is distributed over long periods of time.

There is no definite method of emergence of the larva nor is there any particular place on the shell through which the larva breaks, perhaps by means of a cuticular tooth whose origin could not be determined. Even with a 1.8 mm. homogeneous immersion lens the tooth could not be discerned until 12 to 20 hours before hatching.

Free Living Larval Stage

The sheath of the newly emerged larva, which is wrinkled and closely adherent to the worm, soon absorbs water and becomes taut (fig. 12). The sheath becomes attached to the bottom of the dish or to débris and the worm vigorously lashes from the fixed point which is usually the anterior end, but occasionally may be the posterior end. Fixation appears to be merely a physical phenomenon of surface attraction, for no organ of attachment could be found. Larvae at ordinary room temperatures remain active for 7 to 10 days, while those at 37°C. move very rapidly but cease to move after 48 hours. Larvae at 10 to 18° C. are rather inactive. Fifty first stage larvae have the following measurements: length .175 to .294 mm. (av. .260 mm.); width .013 to .022 mm. (av. 0.017 mm.); length of sheath .255 to .338 mm. (av. 0.31 mm.). The cuticular tooth is 3.75 to 4 micra long.

The internal structure of the larva at this stage is relatively simple. The digestive tract for a third of its length is an undifferentiated narrow tube, that at this point enlarges to form the primitive intestine which constricts to form the non-functioning rectum, located some distance anterior to the tip of the tail. A granular substance of high refractive index called rhabditin by Cobb (1914) is abundant within the intestine. This substance combines with osmic acid to form a permanent black mass and therefore must be some form of a lipoid, an unsaturated fatty acid. A mass of diffuse glandular material surrounds the anterior third of the digestive tract. The cuticular tooth is located at the anterior end and lies obliquely with one end touching the tip of the worm.

First Intermediate Hosts

Since the eggs and larvae adhere to the bottom of the container and are difficult to remove, cyclops were placed in a dish with the larvae. To insure parasite free hosts, many cyclops were collected and kept in an aquarium for approximately a week to permit the development of any natural infection.

The larvae are attached to the substratum by their sheaths and maintain a lashing movement which attracts the cyclops. The capturing and eating of larvae by a cyclops may be readily observed through a binocular dissecting microscope. The crustacean darts toward a larva, hovers over it a moment, eats the worm while vigorously jerking its swimming feet, and then moves toward another victim. The number of worms a cyclops may eat depends to a great extent upon its size and the length of time it is left in the container with the larvae. In order to avoid over infestation the cyclops were permitted to remain with the larvae for only 15 to 30 minutes, when they were removed from the dish by pouring the water into another vessel. Since the larvae are attached to the bottom of the dish, very few go over with the water. More cyclops may be added and the water changed until all of the larvae are ingested. The infected cyclops were fed *Paramecium*. Those cyclops which eat too many worms soon die and are of no value for experimental feeding. Although fifty larvae have been recovered from the body cavity of a dead experimentally infected cyclops, it is obvious that in nature a cyclops has an opportunity to eat only a few larvae.

To determine the fate of the sheath, several cyclops were placed with numerous larvae and were removed after fifteen minutes so they might be examined before all of the larvae had penetrated into the body cavity. When the intestines of the cyclops were dissected out and opened each contained several larvae which had no sheaths, nor were remains of the sheaths found. This examination was performed several times with consistent results. These data suggest that the sheaths have been digested in the alimentary tract of the cyclops, but this supposition is not supported by experimental attempts to dissolve the sheaths from the free living larvae with artificial gastric juice, pancreatic juice, or gastric followed by pancreatic juice. Some mechanical action may play a part in removing the sheath.

After the sheath is removed, the larva with the aid of the cuticular tooth penetrates into the body cavity of the cyclops. Since no glands or ducts lead to the base of the tooth one must assume that penetration by the larva is merely a mechanical process.

Further development of the larva is influenced by several factors, among them the species of cyclops and the number of larvae in the body cavity. Some species, e.g., *Cyclops bicuspidatus*, ingest the larvae but inhibit their growth. Several attempts to develop worms in this species have failed. Growth of the larvae has been observed in *Cyclops leuckarti*, *serrulatus*, *albidus*, and *brevispinosus*. In experiments, *C. leuckarti* appeared to be the most dependable, while *C. albidus*, probably on account of its large size, permitted the growth of the largest number of larvae. Sixteen well developed larvae have been dissected from the body cavity of one *C. albidus*. In general, however, the presence of ten or more larvae in the body cavity of a cyclops precludes the growth of any of the worms. If seven or eight

larvae are present, as many as four may develop, the others remaining alive and active but not growing. Usually if only two or three larvae are ingested, all of them grow to be infective third stage larvae. The fact that many cyclops of the usual host species maintain a few undeveloped larvae within their body cavities for as long as thirty days without growth indicates that unknown factors influence growth of larvae in cyclops. Some of these factors may be nutrition, metabolic activity of the host, and chemical environment.

Rate of larval growth. Many observations form the basis for the data given on rate of growth in larvae. Larvae in the first intermediate host grow at a variable rate. Growth during the first five days is chiefly in diameter, length remaining nearly the same. Thus the diameter at the end of five days is .033 mm. compared with .017 mm. for the free living larvae. The anterior end is truncated and the cuticular tooth is drawn posteriorly and laterally. No definite esophagus is differentiated, but the digestive tract is much broader than in a freshly ingested larva (fig. 11). The sheath with the cuticular tooth attached to its margin is loose around the anterior end of seven or eight day old larvae (fig. 7). The larvae moult between the 10th and 14th day, shedding the tooth with the sheath, and then measure 1 to 1.4 mm. long. Although these worms are infective third stage larvae, they continue their growth. Measurements for 30 worms 20 to 21 days after infection of the cyclops are: total length 1.05 to 1.95 mm. (av. 1.46 mm.), width .018 to .05 mm. (av. .043 mm.), and length of esophagus .313 to .62 mm. (av. .545 mm.). The total length of larvae 30 days after infection is 2.08 to 2.20 mm. Even though there is a difference in size between the two latter groups, the general structures and proportions are nearly the same. The larvae are never encysted in the cyclops but move about in the body cavity (fig. 10).

Morphology of a 2.2 mm. larva. In lateral view, the primitive tri-partite lip is easily recognized (fig. 5). The sub-median papillae are prominent in a dorsal view of the head (fig. 6). The lateral papilla could not be found although many larvae from cyclops and second intermediate hosts were examined under an oil immersion lens. Two regions of the esophagus, muscular and glandular, are well differentiated and two large nuclei near the posterior end of the esophagus are especially prominent. Nerve ring and excretory pore and duct are also visible (fig. 17). The genital primordia consist of only a few cells lying beside the intestine one-third the body length from the posterior end. The intestine has a rich chestnut color because it contains many reddish brown refractory granules. The rectum is functional and several glands are located in the rectal region. On each side of the body posterior to the anus is a small protrusion with four ridges, two of which surround a central pit, while the other two flank two elongate lateral depressions (fig. 9). These structures appear to be strictly cuticular as no sacs

nor internal structures joining them could be discerned. Bastian (1863) in discussing *Dracunculus* larvae described and figured two lateral sacculi which were located about one-third the length of the body from the tip of the larval tail. Jacobson (1834) also in reference to *Dracunculus* larvae mentioned "deux petits mamelons" near the posterior end.

These protrusions are present on all the *contortus* larvae in cyclops and, hence, are not sexual characters. Larvae encysted in the second intermediate hosts and those of a length of 5 mm. or less in the final host have rudiments of these structures, but they were sought for in vain on larvae whose average length was 7.5 mm. Nothing appears to be known concerning these protrusions but it is possible that they may indicate some relationship with an ancestral nematode.

Viability of infective larvae. Well developed larvae are rather resistant to external conditions and remain alive and infective for several days in cyclops after death of the host. Rarely do the worms escape from the cyclops into the water; more frequently they are destroyed by multitudes of protozoa which soon enter the dead crustacean.

Discussion. All of the information concerning the biology of the first intermediate host and its parasite was derived from experimental animals, but there is no reason to doubt that much of the data is directly applicable to natural infestations.

Few known life histories of nematodes have cyclops as their intermediate host. Leuckart (1867: 109-112) in his life history of *Camallanus lacustris* Zoega (1776) formerly called *Cucullanus elegans* Zeder (1800) pointed out that cyclops was the intermediate host of this nematode which lives as an adult in fish. According to Leiper (1907), Fedschenko (1870) demonstrated that the larvae of *Dracunculus medinensis* developed and underwent metamorphosis in the body cavity of cyclops. Leiper (1907) proved that cyclops was an intermediate host of *D. medinensis* when he fed infected cyclops to monkeys and recovered five worms six months later at autopsy. Makin (1927) reported that *Cyclops bicuspidatus* ingest the larvae of *Dracunculus globocephalus* which penetrate into the body cavity of the cyclops, but he said nothing of their development. Thomas (1929) found that *Cyclops brevispinosus* ingests larvae of *Philometra nodulosa* but was unable to report an appreciable growth of the larvae in the body cavity.

Second Intermediate Hosts

Natural infections. Animals with encysted *S. contortus* were collected in several regions in Michigan. Fontinalis Run, near Burt Lake, Cheboygan county, was an area of heavy infestation. Several mud minnows, *Umbra limi*, contained thirty to forty worms each. A high percentage of tadpoles of *Rana clamitans* and newts, *Triturus viridescens*, carried these worms in smaller numbers than the mud minnows. Thirty dragonfly nymphs, prob-

ably of the genus *Sympetrum*, yielded only four *Spiroxys* larvae. Six turtles each had from 25 to 65 adult nematodes. From a pool near Elk Lake five of eight young *Ameiurus nebulosus* were infected, as were the mud minnows. While other small fish as bluegills, *Helioperca incisor*; sunfish, *Eupomotis gibbosus*, etc. from the same pool were negative. From Fife Lake five young *Ameiurus* were positive, each having two to five cysts. One of fifteen dragonfly nymphs contained one larva. A mill race near Ann Arbor yielded nearly one hundred per cent infections in the many *Umbra limi* taken there. Infested young and adult *Rana clamitans* and *Umbra limi* were collected from a pool near the village of Platt. This series of animals covers a wide range, but there are probably many more species which serve as collectors for these worms.

Larvae in fish, tadpoles and newts are surrounded by loose connective tissue which is enclosed in a sac whose walls are only four to six cell layers thick. These cysts are located in the mesenteries of the small intestines. Some of the worms taken from dragonfly nymphs were completely invested in a chitinous spiral cyst which followed the contour of the larva, and the cyst was surrounded by connective tissue and attached to the body wall of the host. Larvae from dragonfly nymphs were enclosed in a cyst composed partially of chitin and partially of connective tissue.

The lengths of the encysted *Spiroxys* in their second intermediate hosts are: in *Umbra limi*, 1.5 to 3.5 mm. (av. 2.76 mm. for 28); in *Rana clamitans* tadpoles, 1.43 to 2.75 mm. (av. 2.12 mm. for 7); in *Triturus viridescens*, 1.78 to 3.3 mm. (av. 2.35 mm. for 6); in *Ameiurus*, 1.75 to 3.18 mm. (av. 2.46 mm. for 2); in dragonfly nymphs, 1.5 to 2.25 mm. (av. 1.9 mm. for 3).

EXPLANATION OF PLATE L

Spiroxys contortus

- FIG. 18. Gubernaculum in *contortus* from British Museum.
 FIG. 19. Gubernaculum in *contortus* from Univ. of Berlin Museum.
 FIG. 20. Posterior end of anomalous male without alae.
 FIG. 21. Adult male reproductive organs, *eja*, ejaculatory apparatus; *ejd*, ejaculatory duct; *s*, spicule, and *sv*, seminal vesicle.
 FIG. 22. Gubernaculum and portion of a spicule, ventral view.
 FIG. 23. Posterior end of male, lateral view.
 FIG. 24. Posterior end of male showing median genital papilla with fibers attached to the cloacal end of ejaculatory duct.
 FIG. 25. Gubernaculum with ligament and muscle attachments, posterior end of the female.
 FIG. 26. Gubernaculum, lateral view with portion of a spicule.
 FIG. 27. Adult male, ventral view showing alae, *g* gubernaculum, *mgp* median genital papilla and the eleven pairs of papillae.
 FIG. 28. *S. contortus*. Section through an encysted larva in the stomach of a turtle. Worm is immediately imbedded in hyaline-like mass which contains a few nuclei, this is surrounded by elongate basophilic cells, and the entire mass is included in a connective tissue capsule.

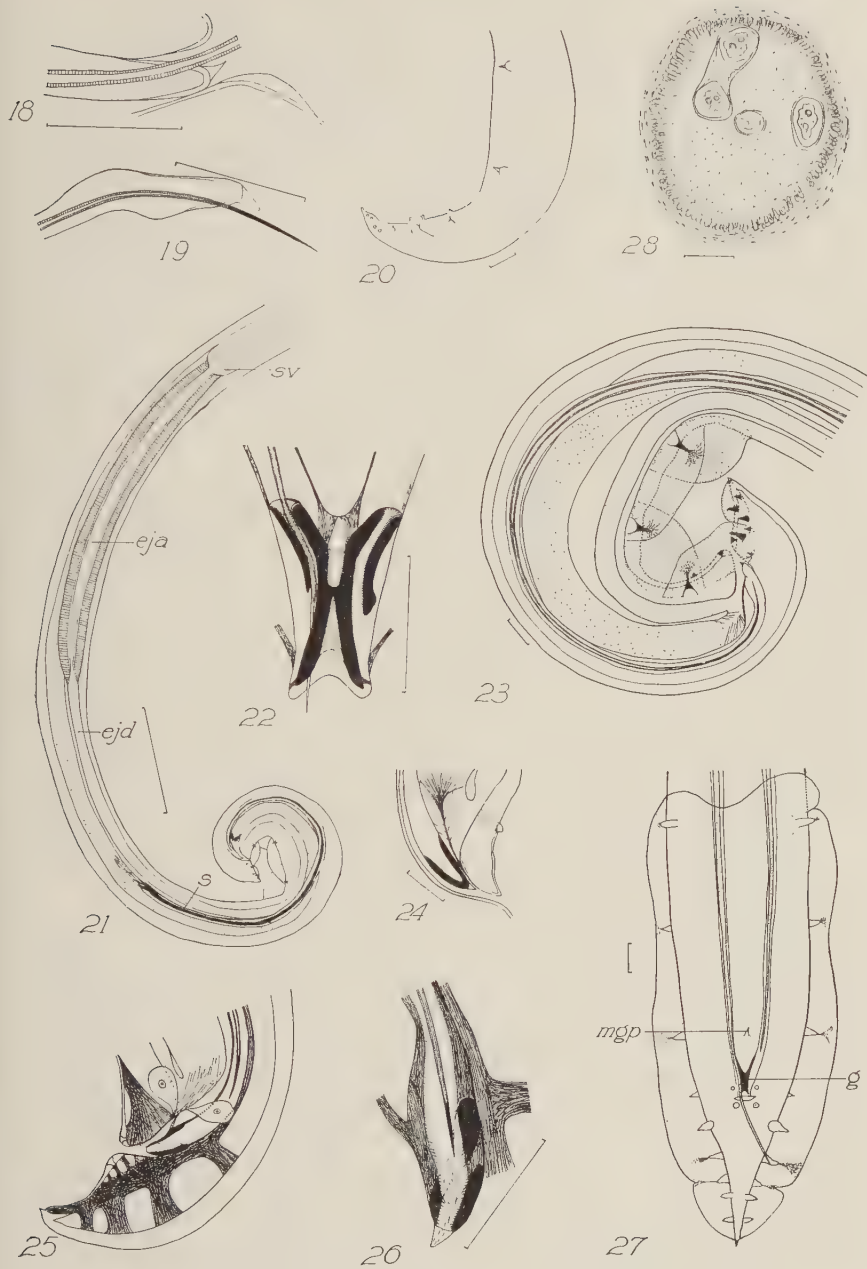


PLATE L

The average length of all these encysted larvae is 2.31 mm. which exceeds but little the average length of 2.12 mm. determined for the few larvae removed from cyclops. This small difference in length leads to the assumption that the worms grow but little in second intermediate hosts. This difference would probably be much less had all the 30 day old larvae reared in cyclops been removed and measured instead of being used for experimental purposes. Furthermore, these second intermediate hosts were infected by ingesting naturally parasitized cyclops. These crustaceans probably permitted the larvae to grow to a larger size than those artificially infected because they carried a lighter infection and lived in a more favorable environment.

Experimental second intermediate hosts. Larvae have been experimentally introduced into snails, tadpoles and Ameiurus.

Snails. Joyeux (1929) reported that certain snails serve as second intermediate hosts for several species of Hymenolepis which live as adults in aquatic birds. Cyclops, Cypris, etc. were the first intermediate hosts. He stated that snails are not entirely vegetarian but also feed on Entomostraca, culicid larvae, etc. and suggested that snails may serve as second intermediate hosts for larval filaria and Dracunculus.

In August, 1931, a snail, *Lymnea emarginata angulata* Sowerby was placed in a watch glass with ten dead cyclops containing well developed larvae. The snail remained in the dish for three days and then was examined. One larva was found in the gizzard wall, one in the liver, one in the esophagus, one in the intestine, and one in the dish after the dissection was completed. All larvae were alive. While this experiment demonstrates the possibility that a snail may serve as an avenue of infection, the fact is that examination of snails from endemic areas have been negative. Moreover, turtles of the genus Graptemys which feed extensively upon snails and other molluscs are lightly parasitized with Spiroxyss.

Tadpoles. The tadpoles which were to be infected with well developed larvae were placed with the known number of infected cyclops in a watch glass covered by screen to prevent the tadpole from escaping. Small dishes were used to permit observation of the cyclops with a compound microscope. The watch glasses were placed in dishes containing water to catch any cyclops washed over by the activity of the tadpoles. If the tadpoles refused to eat, they were fed the cyclops with a medicine dropper.

On October 1, 1931, two tadpoles were fed cyclops containing ten- to twelve-day old worms. The tadpoles died twenty-four hours later and were examined. Several worms were free of the cyclops in the stomach lumen and two larvae were in the act of penetrating through the stomach mucosa. Another tadpole was placed in a dish with twenty-six cyclops containing twentieth-eight infective larvae, September 9. Two days later only three cyclops remained in the dish. These were fed to the tadpole. At autopsy

18 days later, 17 larvae were found encysted in the mesenteries around the stomach and duodenum. A fourth tadpole was given 18 larvae in 14 cyclops, September 12. Thirty-six days after-wards, five larvae were recovered from cysts in the mesenteries. The tadpoles used were not laboratory reared, but were taken from a group, fifteen of which had previously been thoroughly examined and found negative for this worm. For further check, others of the same lot were isolated and examined at the completion of the experiment. All of the controls were negative for Spiroxys.

Ameiurus. A young *Ameiurus nebulosus* was placed in a watch glass with eight living cyclops containing a total of fifteen larval worms. The fish ate only one or two cyclops, the others were fed to the fish with a medicine dropper. Sixteen days later at autopsy, one larva was found boring through the stomach wall. Four other fish from the same collection were negative.

Discussion. Larvae taken from experimentally infected cyclops cannot be distinguished by morphological characters from those taken from naturally and experimentally infected second intermediate hosts. A description of the third stage infective larva has been given under the discussion of the first intermediate hosts.

Larvae in Final Hosts

The only forms of this parasite previously described are the adults or nearly mature worms attached to the stomach mucosa of turtles. However, many interesting and varied stages of the young of this nematode live for a time in the stomach tissues. Some larvae are definitely encysted while others are merely imbedded in the stomach wall. Those that are imbedded may or may not be surrounded by thin layers of proliferated host tissue.

Natural Infections

Encysted larvae. Encysted larvae were found to be especially common in *Sternotherus*. Thirty-one out of fifty-one of these turtles positive for Spiroxys, had the small larvae in the cysts as the only form of this nematode. Many cysts were studied before preservation and again after sectioning and staining. The worms are usually immediately surrounded by a peculiar hyaline-like waxy material. In life this is brown and brittle or flaky. The appearance of this substance in different cysts varied. An attempt will be made to describe the usual forms instead of trying to make a general statement that would include all of the variations. In the early stages of encystment the worms are surrounded by proliferated host tissue rich in nuclei. Then the part of this tissue adjacent to the worm undergoes some form of necrosis, very similar to hyalinization, leaving the nuclei in a homogeneous matrix. Later these nuclei disintegrate and the chromatin diffuses throughout the substance. In most of the cysts stained with Mayer's

haemalum and eosin, this amorphous material was purplish-pink. In others the color varied either toward the blue or toward the pink. When the cysts were stained with Van Gieson's stain the hyaline-like material took only the picric acid and not the acid fuchsin which is a connective tissue stain. Radiating elongate basophilic cells or other host tissue proliferation surrounds this amorphous substance. Several layers of connective tissue fibers surround the entire inner mass and form the outer wall of the cyst (fig. 28). These cysts vary from 0.7 to 1.8 mm. in diameter while the larvae measured 1.2 to 3 mm. (av. 2.14 mm. for 27). The cysts were usually located in the circular muscle layer, but they may also be in the submucosal region. A few have been found attached to the peritoneal surface of the stomach. Some cysts contain degenerating or dead larvae. In other cysts the worms have entirely disintegrated, and the space previously occupied by the larvae is replaced with this amorphous substance. However, not all dead or degenerating larvae are surrounded by this peculiar material. The fate of the larvae in the cysts will be considered after the results of some experimental studies have been discussed.

Imbedded larvae. Most of the imbedded larvae were found in the stomach muscles of *Chrysemys*, only a few in *Sternotherus*. These imbedded larvae are usually larger than those in the cysts. Measurements of 46 imbedded worms are as follows: total length 1.5 to 6.2 mm. (av. 4.00 mm.); esophagus length .8 to 1.6 mm. (av. 1.2 mm.); and maximum width .09 to 0.157 mm. (av. 0.135 mm.). Some of these are invested by a small amount of proliferated host tissue, some are surrounded by muscle infiltrated with eosinophilic leucocytes, and others by muscle tissue without cellular infiltration. The reason for the different reactions of the host tissues toward these larvae is not known.

Threaded larvae. The imbedded worms grow and later usually become threaded into the mucosa of the stomach but occasionally they are attached to the peritoneal side. Twenty-two threaded larvae measure 3.3 to 8 mm. (av. 5.22 mm. in length). In all the examinations made, only fifteen larvae were found threaded into the peritoneal side of the stomach, with the body of the larvae projecting into the body cavity. They measure 2.7 to 6 mm. (av. 4.7 mm.). The third moult occurs when the larvae are within the 4.5 to 6.5 mm. size range. Soon after this moult, the primordial genital cells divide and multiply to form primitive reproductive organs which grow slowly until after the fourth moult, then more rapidly. The fourth moult occurs rather soon after the worms attain the length of 9 to 12 mm. Turtles containing these immature worms were usually collected during the months of April, May, and the early part of June. These larvae grow and become adults. However, before discussing the incidence of the worms in turtle- and the pathological effects of the adults upon the definitive hosts, the results of some experimental studies will be considered.

Experiments upon Definitive Hosts

A variety of experiments have been performed in an attempt to complete the life history with experimental turtles and to increase our knowledge of the larvae in the stomach tissues.

Retention of turtles. Turtles were kept in captivity for certain lengths of time and then examined. The larvae in them were compared in size with those taken from other turtles of the same lot when the experiment was started.

Twelve *Sternotherus* collected in June, 1931 were examined the following March. During the summer these turtles were kept in an open air aquarium on the beach of a lake and fed raw beef and lettuce. In the fall and winter they lived in a large aquarium at an approximate average temperature of 15° to 17°C. The turtles continued to eat until the middle of November. Only one turtle contained an adult *Spiroxys*. The remainder had only encysted larvae. About one-third of the cysts contained degenerate larvae; the others had living worms. A few of the encysted larvae had grown to a length of 3.6 to 4 mm. while others remained at 2 to 2.4 mm.

Three *Chrysemys* were collected from Fleming Creek, near Ann Arbor in October, 1931. Two were immediately examined, one was kept in a warm aquarium (temperature 18° to 22°C.) until it was examined, March 10, 1932. One of the turtles examined in October had several worms in the muscle tissue unaccompanied by host tissue proliferation, and 14 threaded into the serosa. Twelve of these were attached by their anterior ends and two were threaded by their posterior ends. The lengths for both the imbedded and threaded worms were 3.5 to 6.6 mm. (av. 5.0 mm. for 18). The other turtle examined in the fall contained thirty larvae imbedded in the stomach muscle, but no worms threaded into either the mucosa or serosa. Fourteen imbedded larvae measured 3.4 to 6.5 mm. (av. 5.0 mm.) long. The turtle examined in March had larger larvae, 4.3 to 5.6 mm. long (av. 5.0 mm. for 5), imbedded in the stomach muscle and smaller larvae in elongate cysts. These encysted worms were degenerate and were approximately 2.3 millimeters long. It is certain that the turtle did not obtain these smaller ones during captivity but had some different phase of *Spiroxys* not possessed by the other two turtles.

The original purpose of the experiment was an attempt to cause the imbedded larvae to become threaded and to grow. This was not accomplished in spite of the fact that during the five months from October to March, the turtle ate regularly and maintained a considerable amount of activity.

Feeding cysts from turtles. Cysts containing living larvae were teased from the stomachs of *Emys* and *Sternotherus* and fed to other turtles. One *Chrysemys*, after treatment with carbon tetrachloride as described below, was fed 30 cysts extracted from the stomach of *Emys blandingii* on Sep-

tember 29, 1931. This turtle lived in a warm aquarium until February 1, 1932, when it was examined. Five encysted Spiroxys found measured 2.1, 2.1, 2.3, 2.7, and 2.9 mm. respectively. In October, 1931 another treated Chrysemys was fed 85 encysted larvae that had been removed from the stomachs of several Sternotherus. At autopsy, July 7, 1932, only two larvae were found. Since the average length of encysted larvae in Sternotherus is 2.1 mm., it is apparent that those few larvae which reëncyst do not grow and therefore do not reëstablish themselves.

Parasite-free turtles. In July and August, 1931, thirty Chrysemys were given carbon tetrachloride C.P. in an attempt to obtain experimental hosts free of Spiroxys. A dose of 1.5 to 2 cc. per kilogram weight of the turtle was found to be effective and yet cause the death of less than fifty per cent of the animals. This anthelmintic was placed directly into the stomach with the aid of a one cubic centimeter pipette graduated into hundredths. A rubber tube with a pinch clamp and mouth tube was attached to the end of the pipette. After the pipette had been forced into the stomach to the desired depth, the pinch clamp was released and air from the lungs was blown through to force down the anthelmintic.

The turtles usually remained in a stupor for two or three days after treatment, then they gradually regained their normal activity. During the three- to six-day interval they suffered from a rather intense diarrhea. Turtles which were not in good condition or those that were given too much carbon tetrachloride died within twenty-four hours. The turtles that died were carefully examined. In all instances, the worms were either killed in place and removed with the sloughed necrotic mucosa or were killed while free within the lumen. Several turtles were killed and examined 7 to 16 days after the dose was given. It was found that the mucosa of the alimentary tract was regenerated after about 14 days of recuperation. Such turtles have usually regained their normal appetite and may be used as experimental animals. Extreme precaution was exercised in protecting these turtles against accidental parasitism.

This treatment did not kill all of the larvae encysted or imbedded in the stomach. Therefore, ten turtles for controls were isolated and kept free of any avenue of infection. None of the controls had adult Spiroxys, five had only one encysted Spiroxys, and the others contained no Spiroxys. From these data, the assumption was made that an experimental turtle must receive not less than three to four larvae if the results are to be considered significant.

Infection with cyclops. The cyclops were always fed to the turtle with a medicine dropper, because not enough cyclops with infective third stage larvae were ever available to be placed in an aquarium with a turtle for an effective experiment. Four preliminary experiments using turtles 4 to 5 cm. long all showed that the larvae grew to the length of two to four mm.

in these hosts. None of these turtles had been treated with carbon tetrachloride, but they were from a group, eight of which at autopsy had been found to be negative.

Three experiments were performed upon *Chrysemys* treated with carbon tetrachloride. One turtle was fed 30 larvae in *Cyclops brevispinosus* and *C. leuckarti*, September 11, and examined, November 3 of the same year. The autopsy record shows that six larvae were imbedded in the stomach muscles. Four measured 2.2, 3.7, 4.2, and 6.0 mm., respectively. Another *Chrysemys* was fed 18 well-developed larvae in cyclops, October 29. On March 6, only one larva was found bored in between the stomach muscle fibers. This result is not significant since the controls contained one larva plus or minus. The third turtle was given 35 larvae in cyclops, October 12, and when examined, August 18, the following year two adult Spiroxys, 25 and 30 mm. long, were threaded into the stomach mucosa and six active larval Spiroxys were imbedded in the stomach wall. The average length of these larvae is 8 mm. These data prove conclusively that larvae from the infected cyclops undergo direct development into adults in the final host.

Infection with second intermediate hosts. Most of the turtles would not eat when isolated for controlled feeding. For this reason, the fish or tadpoles were forcibly fed to the turtles. In order to expedite the feeding, only the viscera which had been removed from the fish or tadpoles were fed.

Three *Sternotherus odoratus* which had been in captivity since June 1, were given the entrails of five *Umbra limi* containing young Spiroxys on September 21. One was examined 48 hours later, the other two were autopsied seven days afterwards. In all three turtles many larvae were found boring into the stomach mucosa, submucosa, and muscularis mucosa. In the experiments of a week's duration, there was some proliferation of host tissue around the larvae. Two other *Sternotherus* which had been in captivity since June 1, showed no such freshly immigrated larvae.

A treated *Chrysemys* was given 60 larvae from *Umbra limi*, September 6. On October 25, twelve larvae were found in the stomach wall parallel with the muscle fibers, but not encysted. Five of these worms measured in length 4.3, 5.7, 6.6, 7.3, and 7.6 mm., respectively. These larvae have undergone a definite growth for larvae in *Umbra limi* measure about 2.4 mm. long.

Two *Terrapene carolina ornata* were fed the viscera of eight *Umbra limi*, October 28, and were kept in a warm aquarium. The one examined, February 1 contained 25 Spiroxys larvae in the stomach muscles and submucosa. These larvae were not definitely encysted but a thin granular membrane immediately invested the worms and this membrane was surrounded by a small amount of connective tissue. The average length for sixteen of these worms was 4.21 mm. The other *Terrapene* autopsied on March 7 had six worms clearly threaded into the stomach wall, four of them were

attached to the serosa, and two to the mucosa. These measured 3.7, 4.4, 4.7, 5, and 5.1 mm., respectively. Thirty other Spiroxys larvae were in the stomach wall, most of them invested by special host tissue, but they were not encysted. The average length for twenty of these is 4.23 mm. Two Terrapene, fed viscera of eight *Umbra limi* in October, 1931 and examined July, 1932 showed no more development of the larvae than in the two preceding experiments. Furthermore, two treated Chrysemys fed the viscera of eight *Umbra limi* in October, 1931, kept in a warm aquarium throughout the winter and examined August 18, 1932 showed only imbedded larvae averaging 7 mm. in length. Why some of these Spiroxys did not continue growth into adults as did those introduced directly by cyclops is difficult to determine. As mentioned above, all of the numerous larvae examined by me are identical in morphological details. In my opinion, the slowness and uncertainty of the development of all the larvae introduced into the experimental turtles is probably due to some nutritional deficiency.

There seems to be no uniformity or regularity in the factors which determine the reactions of the hosts to the larvae. Some larvae are definitely encysted while others are invested with a small amount of special host tissue. Indeed, some are strictly free in the muscles. The cause of death and disintegration of some of the encysted larvae is also obscure. Furthermore, why should some larvae become threaded into the serosa instead of the mucosa, the habitat of the adult *Spiroxys contortus*?

Encysted Spiroxys have been found in Chrysemys, Emys, Graptemys, Chelydra, and Sternotherus, but they are much more common in the last than in the other genera. Probably Sternotherus is not a particularly good host for the adult parasite. The vitality of the worms is perhaps of importance in determining whether the larvae become encysted. The vitality may depend upon the length of time spent in the second intermediate host or upon the initial development of the worms in cyclops. Attempts to distinguish between those susceptible or not susceptible to encystment by measurements have failed. Continual movement of a larva in the stomach tissue would be an inhibiting factor to encystment.

Some encysted larvae die and disintegrate while others remain active or even grow. Again, size is apparently not a factor because the remains of large worms (4 mm. long) have been found in these cysts. The lack of inherent vitality in the worms seems to be the most important item in the death of these young Spiroxys. Death does not seem to be caused by a lack of food because sections through several of these cysts showed that some of the dead larvae were not surrounded by any amorphous, hyaline-like material or by unusually heavy cyst walls. Furthermore, many of the encysted larvae remain alive for at least a year. In Sternotherus that were kept in captivity for a year some encysted worms had even grown to a length of four mm.

No satisfactory explanation as to why some larvae attach themselves to the serosa and, others of the same size become threaded into the mucosa can be offered. These worms surely migrate back through the stomach wall and take their place in the mucosa, because an adult *Spiroxys* has not been found by me on the peritoneal side of the stomach. However, Morishita (1926) found specimens of *Spiroxys japonicus* attached to the peritoneal side of frog intestines.

Discussion of Life History. The life histories of the nematodes in the super-family Spiruroidea are here summarized from the literature. All have one intermediate host and some may have facultative second intermediate hosts. Those living in terrestrial animals deposit fully developed eggs which do not hatch until after they are eaten by the first intermediate host, some land arthropod. Those living in aquatic animals may deposit fully embryonated eggs which hatch in the definitive host and pass into the water as free-living larvae, e.g., *Echinuria uncinata* as reported by Hamann (1893), or they may be ovoviviparous and the larvae pass directly from the female into the contents of the alimentary canal and enter the water with the feces as in *Camallanus lacustris*. In this super-family the larvae of most species whose life histories are known, spend a period of migration in the tissues of the final host before becoming established as adults.

The feeding habits of turtles are apparently such as to require a regular second intermediate host in the life history of *Spiroxys contortus*. Although the second intermediate hosts are in the strict sense facultative, the fact that turtles are more apt to eat a tadpole or fish than a rapidly darting cyclops strongly suggests that the usual route of infection is through the ingestion of a second intermediate host rather than a first intermediate host, cyclops.

From this survey and discussion of the life history it may be deduced that the life history of *Spiroxys contortus* differs from the usual in these particulars: eggs are deposited in very early cleavage stages and hatch after about a week of incubation in water; larvae have sheaths which become attached to some object and serve as pivots for the actively vibrating larvae; the second intermediate host seems to be more imperative than in the other genera and the time spent in the tissues of the final host is longer than in most of the previously described life histories.

Adults in Final Hosts

Biology. Immature worms threaded into the stomach mucosa are usually found during the spring months. They continue their growth and become adults. Many stages between larvae and adults have been collected from turtles examined in the months of April, May, and June. Adults are seldom found in turtles collected in the Ann Arbor region before June first. When worms are numerous, many have their anterior ends threaded

TABLE I
Stages of Spiroxyes and their incidence in turtles

Species of turtle	Number Examined		Encysted Larvae		Imbedded Larvae		Immature Threaded Worms		Adult Worms	
	Positive	Negative	No. in- fected	Av. No. per turtle	No. in- fected	Av. No. per turtle	No. in- fected	Av. No. per turtle	No. in- fected	Av. No. per turtle
<i>Chrysemys bellii marginata</i>	61	46	6	3	5	24	19	10	33	10
<i>Emys blandingii</i>	9	6	1	25	—	—	2	25	7	3
<i>Emys orbicularis</i>	3	3	—	—	—	—	2	4	1	4
<i>Pseudemys texana</i>	3	7	—	—	—	—	1	8	2	2
<i>Pseudemys elegans</i>	1	15	—	—	—	—	—	—	1	3
<i>Graptemys geographica</i>	4	6	1	4	1	3	2	1	1	2
<i>Clemmys guttata</i>	—	2	—	—	—	—	—	—	—	—
<i>Chelydra serpentina</i>	8	2	2	7	—	—	4	5	5	4
<i>Sternotherus odoratus</i>	51	17	43	7	1	2	12	2	7	1.5
<i>Amyda spinifera</i>	2	5	—	—	—	—	1	4	1	12*
<i>Terrapene c. carolina</i>	—	1	—	—	—	—	—	—	—	—
<i>Terrapene c. ornata</i>	—	10	—	—	—	—	—	—	—	—
<i>Terrapene c. triunguis</i>	—	3	—	—	—	—	—	—	—	—
<i>Kinosternum subrubrum</i>	1	—	—	—	—	—	—	—	1	9

* *Spiroxyes amydae*.

into the bottom of a single ulcer. Of sixty-five adult *Spiroxys* removed from one *Chrysemys*, forty were attached to the base of one ulcer, fifteen to another, and ten to a third. These worms when in 0.75 per cent sodium chloride solution and probably likewise in the stomach of the host, tend continually to coil and twist about each other. If several worms that have been dissected from a turtle stomach are placed in one container, they often aggregate to form a compact knot. The sex ratio is approximately one to one. A total of 188 males and 206 females were found in thirty-one hosts.

Observations indicate that the deposition of the eggs is completed in a short time and that it is more or less confined to one period. The eggs usually adhere to thin membranes sloughed from the stomach mucosa and pass from the host in a group.

Many of the adult worms from turtles collected in late summer and autumn were old. These were often free or mixed with food material in the stomach or intestine instead of being threaded into the stomach mucosa. Such nematodes that have completed their sexual life gradually pass out of the alimentary tract as limp, straight worms.

Pathology. The lesions produced by the adult nematodes are essentially ulcers. When only two or three worms are attached at the same place, little of the mucosa is destroyed and some connective tissue is formed at the base of the lesion. This connective tissue and the adjacent region is infiltrated with lymphocytes and a few eosinophilic leucocytes. In heavy infections chronic or indurated ulcers are produced by clusters of threaded adults. The mucosa about the ulcers is entirely removed and the destruction of tissue often involves the submucosa, muscularis mucosa, and the circular muscle layer. The base of the ulcer is usually filled with a cavernous, firm connective tissue which has replaced portions of the tissue destroyed. The cavernous spaces are rather heavily infiltrated with lymphocytes.

Incidence. All the species of turtles normally occurring in Michigan have been examined except *Clemmys insculpta* (LeConte) and *Chrysemys bellii bellii* (Gray). The former is rare and the Michigan representatives of the latter are present only in the Upper Peninsula, an area from which no collection was made by me. Turtles from a few other states were also examined. The results of examination of turtles are brought together in table I, which does not include turtles used in experiments.

Significance of the table I. The true incidence of *Spiroxys* in *Chrysemys* and *Sternotherus* is in reality greater than indicated in the table since during the early part of the investigation, the presence of larvae in the stomach wall was not suspected. These larvae can be detected only when the stomach tissue is compressed between glass plates. Most of the larvae in *Sternotherus* are encysted. The *Pseudemys elegans* and *Pseudemys texana* examined were not Michigan turtles, but were obtained from commercial firms. The incidence of *Spiroxys* given for them is no doubt lower than the true index.

The *Terrapene carolina ornata* and *T. carolina triunguis* were shipped to me from Oklahoma. The *Kinosternum subrubrum* was collected by me near Houma, Louisiana. Specimens of *Chrysemys bellii marginata* collected near Madison, Wisconsin and Neppel, Washington also yielded *S. contortus*. The *Emys orbicularis* were collected in Dalmatia and sent to America from Germany.

Harwood (1931) found *contortus* in *Pseudemys elegans* from Oklahoma, and (1932) in *P. elegans*, *Deirochelys reticularis* and *Sternotherus odoratus*, collected near Houston, Texas.

IDENTIFICATION

Careful morphological studies demonstrate that *Spiroxys* collected by me from the stomachs of American turtles are con-specific with specimens of *contortus* borrowed from the British Museum and the University of Berlin Museum and likewise with four adult males and four immature worms taken by me from the stomachs of the European turtle *Emys orbicularis*. The most apparent differences between my material and the recognized species *contortus*, are the relative lengths and thicknesses of the head. However, averages for several measurements of various parts of the head in the two groups revealed no real differences. Since none of the descriptions of this species is adequate, it seems desirable to redescribe carefully specimens belonging to the type species of the genus.

DESCRIPTION OF ADULTS

The description is based upon adults of *Spiroxys contortus* from the stomachs of American turtles. Although many specimens were studied, the measurements given for the adults have been made from fifteen specimens of each sex unless otherwise stated.

Specimens have been deposited in the University of Michigan Museum of Zoology, United States Bureau of Animal Industry, British Museum of Natural History, and the University of Berlin Museum.

Adult males and females are rather slender, colorless, semi-transparent worms, two to four cm. long, intestine distinctly brown or approaching black in some individuals. The oral opening is surrounded by two tri-lobed lips with a rather thick cuticular lining which in each median lobe projects anteriorly to form a blunt tooth (fig. 3). Each lip bears two submedian and one lateral papillae (fig. 2). The former are easily recognized, especially when the head is viewed from its dorsal aspect. The lateral papilla, however, even with extremely careful manipulation of the substage condenser is discerned in only an occasional specimen. The anterior margin of the dorsal and ventral lobes is usually quite angular but may be rounded. Thus, it is impossible to use the shape of these lobes as a diagnostic character, as did Morishita (1926). The vestibule has distinct cuticular prominences and

indentations. A definite cuticular collar bounds the posterior margin of the head. A tri-lobed cuticular support for each of the two lips arises from each arch of the collar (fig. 4). Two prominent cervical spines and one small inconspicuous lateral spine are located on each side of the collar (fig. 2), and in particular on the cross pieces connecting the arches (fig. 4) not on the arches of the collar. The large spines, when viewed dorsally, form two more or less distinct tooth-like projections on the anterior margin of the dorsal and ventral walls of the vestibule (fig. 3).

The nerve ring is 0.47–0.67 mm. from the anterior end. Masses of nerve ganglia are immediately anterior and posterior to the nerve ring. The excretory pore, 0.525–0.75 mm. from the anterior end, is the opening of a duct leading into a small sac ventral to the esophagus. The single dorsal and ventral cervical papillae are small and are located 0.8–0.9 mm. from the anterior end of the worm. The esophagus in gross structure is undivided and gradually widens posteriorly, but histologically it may be divided into an anterior muscular part, a median portion composed of spherical glands supported by small amounts of connective tissue, and a posterior part which has numerous fibrous trabeculae separating the diffuse glandular constituent (fig. 8). The posterior end of the muscular part of the esophagus and the posterior end of the esophagus are 0.375–0.435 mm. and 1.5–5.7 mm., respectively from the tip of the head. Two large nuclei near the posterior end of the esophagus are difficult to detect in the adult because of the glandular material. Three small valves guard the entrance of the esophagus into the tessellated intestine. These polygonal boundaries are the outlines of elongate intestinal cells which rest upon a cuticular basement membrane. The intestinal lumen is often filled with short bacilli which, in some instances, destroy the elongate intestinal cells and leave only the cuticular basement membrane intact. The rectum of the female and cloaca of the male have two large ventral and one large dorsal unicellular glands (figs. 16 and 25). There are also numerous smaller glands in this region. The tail in both sexes is rather short and ends in an abrupt, sharp, conical tip. The transverse cuticular striations which extend the entire body length are rather close together. The distances between the striations at the anterior end are approximately three to four micra, while those for the middle and posterior regions are about five to six micra.

MALES

Alae are well developed and when stained with paracarmine are distinctly mottled, the more or less homogeneous matrix interspersed with trabeculae of coagulated liquid within the alar lamellae. In some cleared unstained specimens the alar content is alveolar and reticular. The reticular or fibrous elements arise principally from the regional papillae. The alae meet ventrally anterior to the anus and form a vesicular swelling (fig. 23).

One adult specimen has no alae, but the papillae are well developed and normal in number (fig. 20). This condition is probably an anomaly rather than an instance of extreme variation.

Papillae. There are eleven pairs of papillae, four pre-anal and seven post-anal. The seventh and eighth pairs from the posterior end are sessile and located immediately posterior and anterior to the anus respectively. The remainder are pedunculate and support the alae. Seurat (1918) mentioned a pair of adanal lateral genital papillae in his description of *S. contortus*. Since he described only eleven pairs in all, this adanal lateral pair may be one of the eleven pairs normal to the genus, and is possibly pair number six.

Median genital papilla. In addition to the eleven pairs of papillae previously known, there is a single median genital papilla 0.108 to 0.196 mm. (av. 0.127 mm. for 5) anterior to the anus. This papilla, quite prominent in some specimens, buried in the cuticula in others, is connected to the opening of the ejaculatory tube at its junction with the cloaca, by strands of fibers (fig. 24) which perhaps are nerve fibers. If so, they probably transmit impulses to the mechanism regulating the opening of the ejaculatory tube, when the median genital papilla is stimulated during copulation.

Spicules. The spicules are long, slender, cylindrica, without a keel, distinctly transversely striated, ending in sharp points. In life and in whole mounts they appear hollow, but sections through the spicules demonstrate a precipitated protein which signifies that the spicules are filled with a liquid in the living animal. When retracted they are enclosed in a somewhat cuticular spicule sheath. Although most of the previous workers on this species state that the spicules are sub-equal my data prove them to be equal to sub-equal. Twenty pairs of spicules measure 1.85 to 3.5 mm. (av. 3.0 mm.) in length. The spicules of twelve pairs were of equal length, those of eight pairs not equal. The differences in length for the latter were .10 to .29 mm. (av. .19 mm.).

Gubernaculum. Males of this species possess a colorless gubernaculum which in sections is seen to be composed of dense cuticular material (considering cuticula in the strict sense). When the posterior ends of living males were studied between two glass slides, the tip of the gubernaculum was repeatedly thrust out the anus and retracted. In ventral view the gubernaculum as a whole is approximately Y shaped with the stem of the Y directed toward the anus. The dense cuticular part of the gubernaculum is roughly X shaped (fig. 22). The diverging anterior prongs have curved ventral grooves. The spicule sheaths parallel the sides of the gubernaculum and merge with the latter at the posterior end. A lateral view of the structure resembles the end of a scythe with a sharp cuticular tip at the end, on the concave side (fig. 26). A ligament from the ventral side is attached to the cloaca (fig. 25). Many muscles attached to the dorsal side of the guber-

naclum extend to the body wall. Measurements of the gubernaculum from the ventral aspect are: width at the posterior end .033-.055 mm., width at the anterior end .09-.10 mm., and total length .150-.171 mm. Measurements from the lateral aspect are: length .140-.164 mm., and thickness .049-.063 mm.

Only two previous workers have discovered a gubernaculum in this genus, Seurat (1918) in worms identified by him as *S. contortus* from an Algerian turtle *Clemmys leprosa*, while Walton (1927) mentioned a poorly chitinized accessory piece in *S. constrictus* (Leidy 1856) Walton. Assuming the accuracy of Seurat's description of the worm there is reason to believe that his material was not *S. contortus*. If this be true no other worker apparently has described a gubernaculum for this species. A gubernaculum was also found by me in specimens of *contortus* from the British Museum (fig. 18), from the University of Berlin Museum (fig. 19), from Rice Institute, and from the stomach of *Emys orbicularis* shipped to America from Europe, in specimens of *S. constrictus* from the Philadelphia Academy, in *S. amydae* from the United States Department of Agriculture, Rice Institute and the author's material, and in *S. allegheniensis* from the Ward collection at the University of Illinois. The specimens of *contortus* from the British and Berlin Museums are so old and so poorly preserved as to prevent the making of a detailed drawing of the gubernaculum.

Reproductive organs. The testis is long, tortuous, joining the seminal vesicle 10 to 15.2 mm. anterior to the anus. The vesicula seminalis, a rather straight, thin-walled sac, 4.6 to 6.2 mm. long and full of sperm, empties into the thick-walled ejaculatory apparatus which is 2.3 to 3.4 mm. long and relatively free of sperm (fig. 21). The slender ejaculatory tube has a thin wall with many pyriform cells projecting into its lumen. It is 3.4 to 5.4 mm. long, and empties into the cloaca (figs. 21 and 23). Spermatozoa in the posterior end outside the sperm duct and cloaca of whole worms as well as in sections indicate an opening into the so-called body cavity. Sagittal sections through the posterior end revealed a diffuse glandular substance in the region posterior to the anus.

FEMALES

Two cuticular prominences guard the opening of the vulva which is just posterior to the middle of the body. The vagina is directed anteriorly, thick-walled, muscular, annulated, and 0.72 to 1.66 mm. long. After a distinct curve, it joins the two uteri. The vagina has a rather heavy cuticular lining but the uteri are lined with thin crenated cuticular membranes. In a mature or gravid female each uterus has two separate thin-walled pouches connected by a slender intra-uterine tube (fig. 13). Intra-uterine tubes have thick walls and usually are slightly coiled. Various parts of the uteri measure: first pouch anterior, length 1.36-1.51 mm., width 0.20-0.21 mm.;

intra-uterine tube anterior, length 0.75 mm., width .075 mm.; second pouch anterior, length 5.2–6.1 mm., width 0.272 mm.; first pouch posterior 1.29–1.51 mm.; intra-uterine tube posterior, length 0.76 mm., and second pouch posterior, length 5.53–6.3 mm., width 0.30 mm. Morishita (1926) stated that the vagina of *S. japonicus* divides into the superior and inferior ovijectors, which in his illustration (fig. 23) are long tubes (about 1.5 mm. that join the uteri. From his description and illustrations it may be inferred that each uterus has only one pouch. There are no ovijectors in the female genital organs of *S. contortus*. The second uterine pouches join the oviducts which lead into the long and much coiled ovaries. The caudal papillae in the female reported for *S. gangeticus* by Baylis and Lane, and *S. annulatus* by Baylis and Daubney are also present in *S. contortus* (fig. 16). These consist of two dorso-lateral papillae on the caudal part of the female, located about mid-way between the anus and caudal tip. In ten worms the distance from the caudal papillae to the posterior tip measured 0.225 to 0.27 mm. (av. 0.238 mm.).

SUMMARY

1. The life history of *Spiroxys contortus* has been studied in detail, the first described for any member of the genus.
2. Eggs are deposited in the one- to two-celled stage and hatch after approximately one week of incubation in water.
3. The newly emerged larvae are sheathed and actively vibrate in the water with their sheaths attached to some object as a pivot.
4. These larvae are ingested by cyclops and develop to the third infective stage in the body cavities of *Cyclops leuckarti*, *albidus*, *serrulatus*, and *brevispinosus*, which serve as the first intermediate hosts.
5. Second intermediate hosts found naturally parasitized with *S. contortus* larvae are: mud minnows, *Umbra limi*, bullheads, *Ameiurus nebulosus*, tadpoles and adults of *Rana clamitans*, larvae and adults of newts, *Triturus viridescens*, and dragonfly nymphs probably of the genus *Symptetrum*. Tadpoles and *Ameiurus nebulosus* have been experimentally infected by feeding them on artificially infected cyclops.
6. Several species of turtles have been found to be naturally infected with Spiroxys, not only by the adults, but also with encysted and imbedded larvae in the stomach tissues, and young worms threaded into the stomach mucosa. Internal factors regulating the different relations of the larvae to the stomach tissues of the final host are not well understood and merit further investigation.
7. Turtles have been experimentally infected with Spiroxys by feeding them either cyclops with third stage infective larvae or tadpoles and *Umbra limi* with encysted larvae.
8. Carbon tetrachloride in doses of 1.5 to 2 cc. per kilogram weight of

the animal was used to advantage in removing Spiroxys from turtles to be used as experimental hosts.

9. The adults were carefully studied and new morphological characters established for the species are: a small spine on each lateral surface of the cuticular collar, gubernaculum and median genital papilla in the male, and two dorso-lateral papillae on the tail of the female.

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